Tag No.

Purpose: Continuation of pg 124 - 125

Amplified linearized pcr / xmn 1 using 2 different new sets of primers

36

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Tried with Taq and Taq + S.V

titrated Mg 1.5, 2.0, 2.5, 3.0 mM

200 µg dNTP
.4 µM primer
1 U/µg enzyme - Taq
25 fg template

cycling: 94° 30" 1
(94° 30 s / 60° 1') 30

product = 1275 bp.

prepare 10x of each: Taq / #3 Taq + SV / #3
" / #2 " / #2

	10x	1x
H2O	338	330 µl
10x buffer	50	
dNTP	10	
Mg	—	
primer 1	20	
2	20	
Template	10	
enzyme	2	— 10 µl Taq + SV
	450	

1.5	2	2.5	3 mM
7.5	10	12.5	15
42.5	40	37.5	35
50			

45 µl / RX added 5 µl of Mg dil. conc.

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	12/9/94	Recorded by K. Sitaraman	12/9/94

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Project No._____

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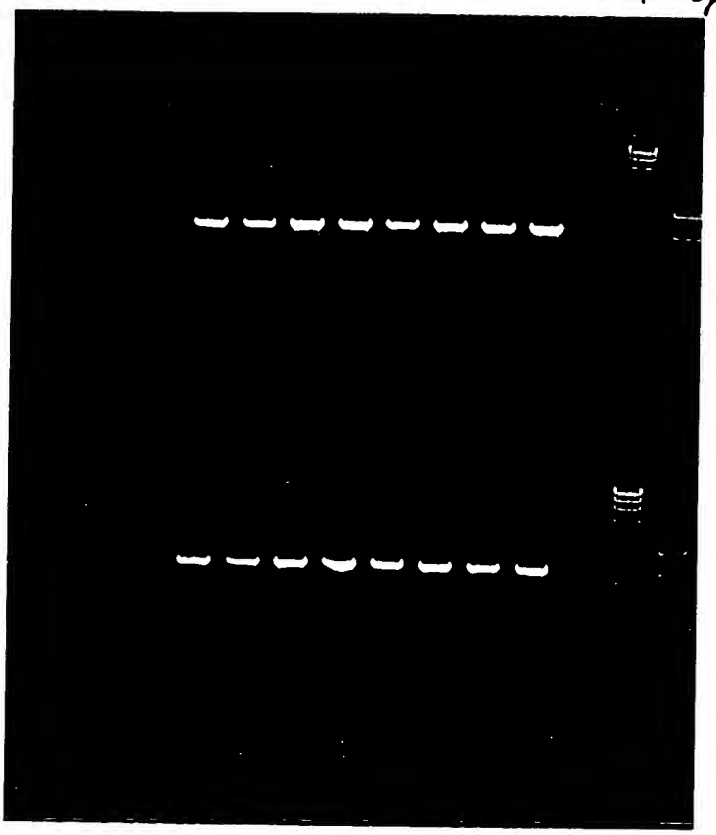

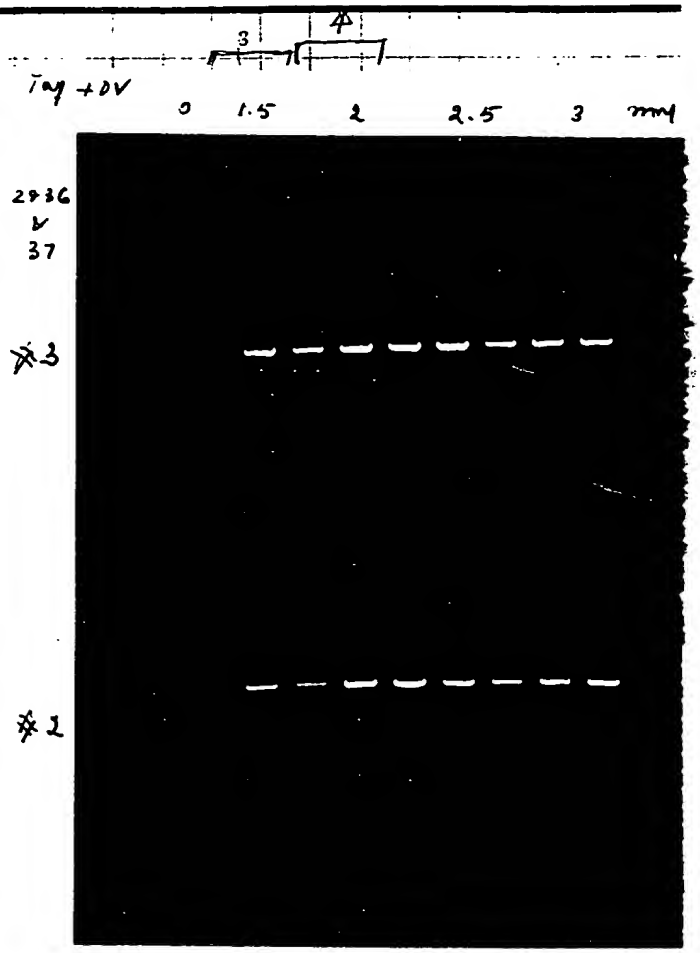

2936 V 37

~ 1275 bp product.

Both primers set work with Taq as well as Taq + D

a bit of mispriming still - has to be gel purifie

good range of Mg tolerance.

pooled ① Taq 1.5 mM Mg separately) with #3 set of primer

② 2.0

③ Taq + DV 1.5 "

④ 2.0 "

and pooled in ethanol pp

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Witnessed & Understood by me,	Date 12/19/94	Invented by	Date 12/8/94
		Recorded by K. Sivaraman	

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pooled RK from two tubes (duplicate of same) together = 30 µl +
made up the volume to 100 µl 30 µl

added equal amount of phenol: chloroform: IAA mixture
removed the aqueous phase after a spin of 5'
phenol extraction again.

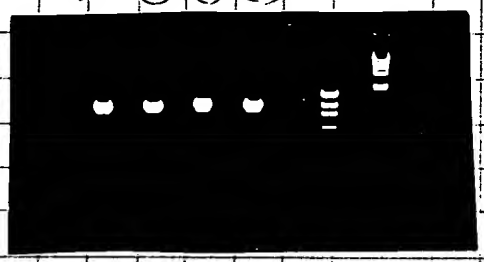
added 0.5 volume of 7.5 M ammonium acetate and 2 vol
of ethanol, added also a µl of Dextran T 500.

left at −20°, 1.5 hr

spun down, removed ethanol, washed the pellet with
70% ethanol, spun down, removed the supt.
spun again to remove the residual ethanol
pellet visible. Vacuum dried 5'
resuspended in 17 µl of TE. removed 2µl for gel

for 15 µl added
10.5 " H_2O
3.0 " 10 x buffer ←
1.0 " Hpa III (7U/x)
0.5 " Aat II (24U/x)

30 µl incubated at 37°, 2 hr.



phenol extracted product seems to be around ~ 150 - 200 ng/x 2
= 75 ng/x

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Read & Understood by me,	Date	Invented by	Date
[signature]	12/1984	Recorded by K. Silverman	12/5/84

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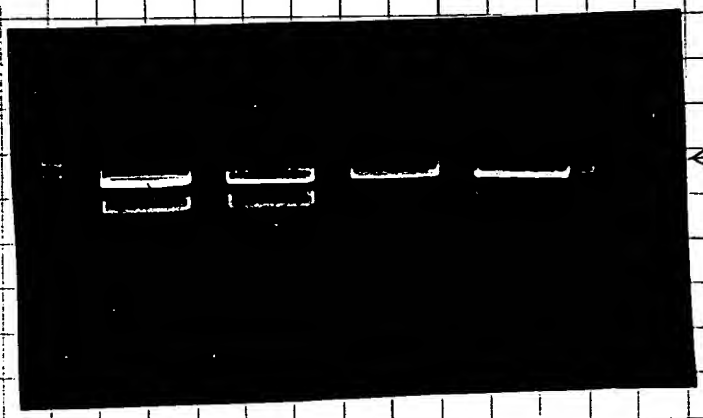
- 15 µl of left over phenol chloroform extracted + ethanol ppted insert + were cut with Bal II and Pst 3 in NEB buffer 4 for 2 hrs at 37°

- Run on 1% gel and transferred to DAEA paper and eluted the fragments in high salt buffer, one ltr of 1M NaCl, 0.1M Tris pH 8.0, 5mM EDTA

- spun down the elution buffer, added 50 µl 3mM of ↓ centrifuged, pooled the elutions, ethanol ppted in ~ 150 µl ~ 500 µl
in the presence of 1 µl of Dextran T- 40.

- left at -70°, 2 ½ hrs, resuspended in 15 µl of left ethanol dried in TC.



← excluded to serve as insert

875

loaded ~ 75 ng × 15 µl = 1125 ng (1275 bp)
= 772 ng (875 bp)

~ 50% recovery = ~ 386 ng / 15 µl
= ~ 25 ng / λ

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Witnessed & Understood by m ,	Date 12/14/94	Invented by	Date 12/12/94
		Recorded by K. Sitaraman	